

Escalation of Aggressive Interactions During Staged Encounters in *Halictus ligatus* Say (Hymenoptera: Halictidae), with a Comparison of Circle Tube Behaviors with Other Halictine Species'

N. Pabalan,¹ K. G. Davey,² and L. Packer^{2,3}

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Circle tube experiments on the primitively eusocial bee, Halictus ligatus, were performed for a variety of combinations of caste and size. Push, Lunge, and Back without reverse behaviors enabled us to determine the dominant individual in most comparisons. Behavioral differences were readily detected within the first 15 min except for different-size forager–forager and same-size foundress–foundress pairs, for which 30 min of observations was required. In same-size forager–forager pairs, no differences in behaviors between individuals were detected even after 90 min. In extended observations, decreases in the frequency of the mild dominant behaviors were accompanied by a switch to the highly aggressive mandibular hold on the neck, particularly in different-size foundress–foundress and gyne–gyne pairs. Three workers were killed by their own queen as a result of this escalation of aggression. We discuss caste-based differences in the circle tube setting in terms of behaviors expected under more normal conditions and compare our data with published results from other species.

KEY WORDS: aggression; eusociality; queen; worker; sweat bee; dominance.

¹Department of Laboratory Medicine and Pathobiology, Banting Institute, University of Toronto, 100 College Street, Toronto, Ontario M5G 1L5, Canada.

²Department of Biology, York University, 4700 Keele Street, Toronto, Ontario M3J 1P3, Canada.

³To whom correspondence should be addressed. e-mail: bugsrus@yorku.ca.

INTRODUCTION

Eusociality represents the most complex form of social behavior (Wilson, 1975). [For summaries of recent terminological debate, see Costa and Fitzgerald (1996) and Weislo (1997)]. It has been defined *sensu* Michener (1974) as having both reproductive division of labor and overlapping generations so that offspring assist parents in brood care. These traits distinguish functional groups based on reproductivity and nest duties (Michener, 1974). Inhibition of ovarian development in nonreproductive females of advanced eusocial insect colonies (i.e., those with extreme queen-worker dimorphism) is accomplished mainly by pheromonal secretions of the reproductive individual(s) (Wilson, 1971; Fletcher and Ross, 1985; Bourke, 1988). This type of chemical control is efficient in large colonies (Michener, 1974, 1990; Wheeler, 1986) in which the reproductive individual (queen) is the sole egg-layer and is dependent on nonreproductive individuals (workers) for her daily existence (Winston, 1987). In contrast, in primitively eusocial species, suppression of ovarian development in workers is frequently a result of aggressive behavior (i.e., nudging, butting) by the principal egg-layer (Free *et al.*, 1969; Wheeler, 1986; Michener, 1990; Roseler and Van Honk, 1990). In primitively eusocial Hymenoptera, aggression is one of the most distinct behavioral attributes of the queen and may serve to establish dominance hierarchies in small colonies (Michener and Brothers, 1974; Breed and Gamboa, 1977; Michener, 1990). Workers may still escape dominance assertion by the queen by avoiding her and workers may lay eggs (Buckle, 1982). However, differential oophagy by the primary reproductive may prevent successful development of worker-laid eggs (Brothers and Michener, 1974; Packer and Owen, 1994). This egg-eating activity appears to bolster the queen's reproductive dominance (Kukuk, 1992a).

In primitively eusocial species of the family Halictidae, gynes are females that overwinter and become foundresses by initiating nests in spring. The nests are frequently found in aggregations which may be quite dense (Batra, 1966; Kukuk and Decelles, 1986; Knerer, 1992). Some nests may contain more than one foundress and in these cases, one individual becomes a guard. Guards are the reproductively dominant individuals and the other foundresses assume foraging duties (Packer, 1993). Multifoundress associations are uncommon in comparison to single foundress nests in most species (Packer, 1993). As single foundress nests are left unguarded during foraging trips and usurpation attempts may occur, conflict over nest ownership (upon return from foraging flights) may arise in these nest aggregations (Kaitala *et al.*, 1989). The duration of such conflict may be brief (5 s or less) or may be extended as in *Lasiogossium malachurum*, where prolonged fights between intruder and nest owner last from 10 to 26 min, involve nudging and

mandibular aggression, and can result in the damage to, or even the loss of, legs and other body parts (Smith and Weller, 1989).

In established nests, workers may directly compete with the queen and with each other for oviposition (Packer, 1987). Nests containing these matrilineal colonies are usually guarded by a worker (Michener, 1990). In addition to competition for egg-laying, agonistic behavior between guards and nonnestmate foragers may occur during nest defense. The guard blocks the nest entrance with its abdomen or assumes a "C" posture with head and sting extended toward the intruder (Bell and Hawkins, 1974; Smith and Weller, 1989). These behaviors are accompanied by thrusts (head or abdominal) to eject the intruder. Such aggressive interactions contrast with the ease with which nestmate foragers are permitted to reenter the nest. In *L. zephyrum*, admittance of nestmates is based on familiarity and genealogy (Kukuk *et al.*, 1977; Breed *et al.*, 1978; Greenberg, 1979; Smith, 1987; Kukuk, 1992b) as well as age (Bell *et al.*, 1974) and is mediated by odors (Bell, 1974; Bell *et al.*, 1974; Greenberg, 1979; Buckle and Greenberg, 1981; Smith, 1983), which would seem to be derived from macrocyclic lactones (Smith *et al.*, 1985) produced by Dufour's gland (Duffield *et al.*, 1981; Smith and Wenzel, 1988).

Behavioral interactions in nests of ground nesting halictine species are not directly observable under natural conditions (Michener and Wille, 1961; Batra, 1964; Breed *et al.*, 1978). Colonies of bees can be maintained in soil between panes of glass in the laboratory (Kamm, 1974; Brothers and Michener, 1974; Michener and Brothers, 1974), but these artificial nests do not allow observations of repeated interactions between two bees in the absence of influences from other individuals. The circle tube, a circular cylinder of clear plastic, has permitted repeated observation of behavioral interactions between individuals under laboratory conditions (Breed *et al.*, 1978; Kukuk and Decelles, 1986; Smith and Weller, 1989; Kukuk, 1992b; McConnell-Garner and Kukuk, 1997; Weislo, 1997; Paxton *et al.*, 1999). Two bees placed in the tube, the diameter of which approximates that of the nest burrows, repeatedly interact, allowing systematic observation of interactions between individuals of known caste (Breed *et al.*, 1978; Kukuk and Decelles, 1986; Smith and Weller, 1989).

Comparisons of the frequency of interactions among bees between laboratory observation nests and circle tube experiments in *L. zephyrum* and between the tubes and nest entrances in *L. malachurum* and *L. pauxillum* suggest that behaviors of the bees in the circle tubes are similar to those that occur in the nest (Kukuk, 1992b; Smith and Weller, 1989).

The duration of observation of bees in past circle tube experiments ranged from 10 to 15 min in species with a diversity of social behaviors: solitary [*L. platycephalum* (McConnell-Garner and Kukuk, 1997) and *L. figueresi*

(Wcislo, 1997)], communal [*L. hemichalceum* (Kukuk, 1992), *Andrena scotica* and *Panurgus calcaratus* (Paxton *et al.*, 1999)], and primitively eusocial [*L. zephyrum* (Breed *et al.*, 1978; Kukuk and Decelles, 1986), *L. malachurum* and *L. pauxillum* (Smith and Weller, 1989)]. All of these bees are halictines with the exception of *A. scotica* and *P. calcaratus*, both of which are in the family Andrenidae. The short observation period was long enough to allow the experimenter to determine which bees were more aggressive during the interactions. In *L. malachurum*, queen and worker behaviors did not change over time when their stay in the tube was extended to 20 min (Ayasse, personal communication). There was likewise no temporal change in activity within the 10-min trials among *L. zephyrum* guard pairs, and although interactions for this caste pair type were prolonged to 30 min, the interactants never passed one another (Breed *et al.*, 1978).

Here, we investigate temporal changes in circle tube behaviors of the primitively eusocial sweat bee, *Halictus ligatus*. Detailed observations of social interactions among castes of this species were undertaken (i) to establish the time required for us to determine dominance by one bee over another during the encounters, (ii) to determine which behaviors most readily enable us to recognize dominance and to see if these vary depending upon the castes of the paired bees, and (iii) to determine whether terminating circle tube experiments after about 15 min (the standard duration for these investigations) results in any significant loss of information.

MATERIALS AND METHODS

Collection of Samples

All samples of *Halictus ligatus* were obtained within and around the campus of York University, Toronto, Ontario. Overwintered foundresses were netted from flowers and at nest aggregations in late May to June during their nest initiation phase. Most foragers were obtained in the same manner between mid-July and mid-August. During this period, nests that contained older queens and younger foragers were excavated. Guards that were observed at the nest entrances for several minutes were removed from their nests with forceps. Gynes were netted over the aggregations or obtained from excavations in the latter part of August to early September (i.e., prior to overwintering). Gynes were identified by the lack of wear on their wings and mandibles (Packer, 1986b); workers caught over the aggregation during this time period had worn wings and mandibles, and they were also smaller.

Circle Tube Experiments

Bees were brought to the laboratory and placed in a refrigerator for approximately 0.5 h at 10°C, after which their head widths and wing lengths were measured and their wing and mandibular wear was assessed. A 20-cm-long piece of clear plastic tubing of the appropriate diameter (Fisherbrand 14-169-7B: 0.5-cm inner diameter) was fashioned into a circle. Since used tubes may contain odors from former occupant bees (Smith and Weller, 1989), each circle tube was used only once, then discarded. A total of 230 bees (115 pairs) was used throughout the series of experiments from May to September 1992.

The paired bees stayed in the circle tube for 75 to 120 min [each termed a trial (see Breed *et al.*, 1978)]. Throughout each trial, interactions between the paired females were continuously monitored. If the bees were inactive for more than 5 min beyond 75 min in the tubes, the experiments were terminated. Bees were used in only one trial, after which they were removed from the tube and placed in buffered formalin (Humason, 1973). The ovaries were removed and processed into whole mounts. Ovarian development was assessed using the index of summed oocyte development (ISO), which is the sum of the stages of development of the most developed terminal follicle in each of the six ovarioles. For an explanation of the stages of oocyte condition in halictine bees, see Goukon *et al.* (1988).

Durations of circle tube experiments for this species were considerably longer than in previous studies of *Lasioglossum* species (Breed *et al.*, 1978; Kukuk and Decelles, 1986; Smith and Weller, 1989; Kukuk, 1992b; McConnell-Garner and Kukuk, 1997; Weislo, 1997) because preliminary observations indicated temporal variation in some behaviors, and in some cases the bees engaged in sustained aggression only after approximately 45 min in the tube (see Results). Interacting bees that engaged in such behavior were observed through a Wild M5A Stereomicroscope at a magnification of 60×. The tape-recorded observations were later transcribed.

Choice of bees for pairing was based mainly on caste/subcaste and size (the latter based on head width). Studies of *L. zephyrum* indicate that guards, foragers, and queens are behaviorally differentiated in circle tube encounters (Breed *et al.*, 1978) and that size and age also influence behavior (Michener, 1977, 1990; Michener *et al.*, 1971). Consequently, we used spring foundresses, summer queens, autumn gynes, guards from nest entrances, and foraging workers. Different-size pairs were those in which the head width of one bee differed from its counterpart, while same-size pairs were those in which both bees had the same head width (Fig. 1). Individuals in pairs with the same head width were distinguished from one another by differences in wing wear. The

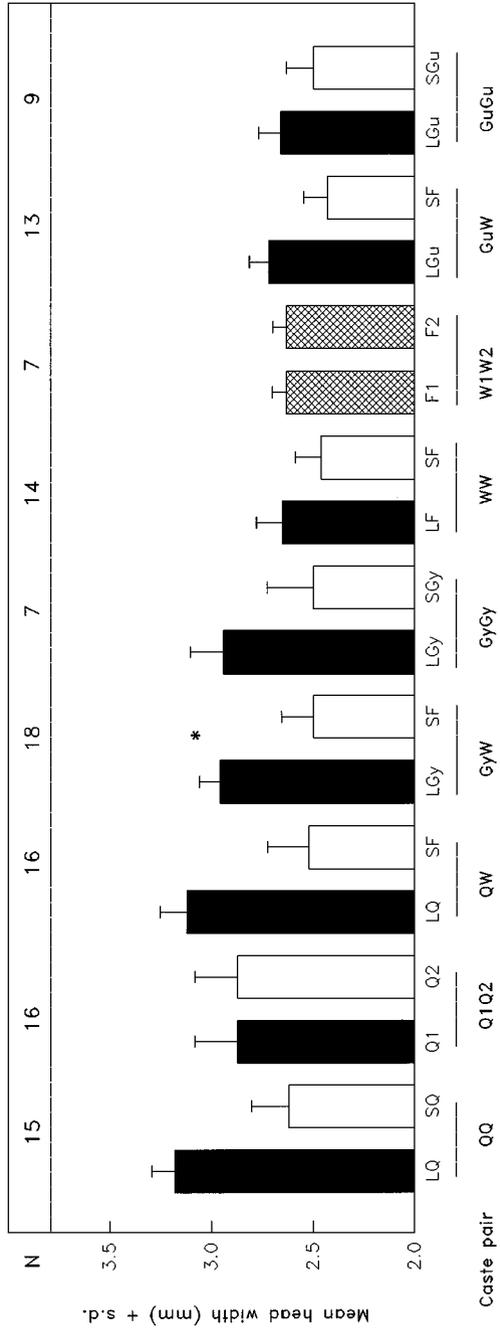


Fig. 1. Size comparisons of caste members of *Halictus ligattus* paired in circle tubes. Measurements were made to the nearest 0.05 mm.

number of pairs for each caste combination was limited by the availability of samples to those shown in Fig. 1.

The method by which most of the samples were collected (netting) did not permit us to ascertain whether the paired bees were nestmates or not except in (i) queen–forager pairs where both members came from the same nests and (ii) guard pairs all from different nests. The wide area from which the bees were netted suggests that there is a very low probability of foragers being nestmates given an average of two or three workers per nest, guards included, during the summer foraging period (Packer, 1986b; Packer and Knerer, 1986).

The Behavioral Repertoire

Behavioral patterns described here were modified from those of Bell and Hawkins (1974) obtained from observation nests (Kamm, 1974) and the circle tube observations by Breed *et al.* (1978) of *Lasioglossum zephyrum*, Packer (1986a) from observation nests of *H. ligatus*, Smith and Weller (1989) from their nest and circle tube observations of *L. malachurum* and *L. pauxillum*, and Kukuk (1992b) from her observations of *L. (Chilalictus) hemichalceum*. The nine behaviors were grouped into three broader categories which we have modified from Kukuk and Decelles (1986).

Aggressive Behaviors

1. PUL (Push and Lunge): One bee pushes another with the end of its abdomen or lunges forward with mandibles open. Although these behaviors appear discrete, the patterns with which they occurred were similar in all cases and so we combine them in the analyses that follow. PUL is considered to be an aggressive behavior likely to be exhibited predominantly by the dominant bee in a pair.

2. MHN (Mandibular Hold on Neck) (Fig. 2): This is a violent and sustained behavior where the mandibles of one bee are clamped around the neck of the other, which is dragged through the circle tube. Clearly this is an aggressive behavior likely to be demonstrated by dominant bees.

3. MHA (Mandibular Hold on the Appendages): The mandibles of one bee grasp a wing, antenna, or limb of the other. This may occur offensively, where two bees meet and one of them bites the appendage of the other. It may also occur defensively where a bee throttled by MHN displays MHA. Because of its use in both offensive and defensive situations when a clearly subordinate bee is being throttled, this behavior is not considered to be necessarily associated with the dominance of one individual over another.

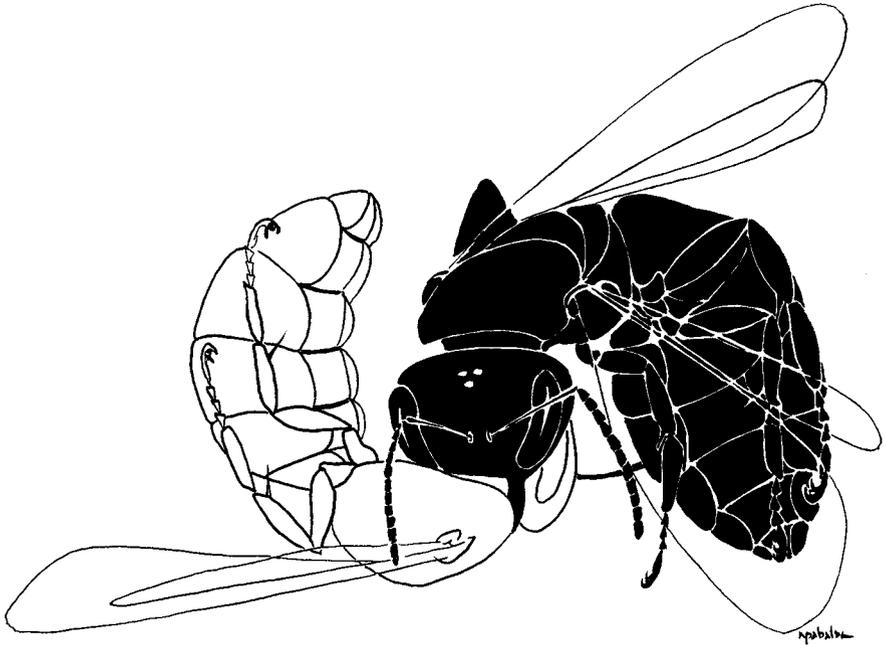


Fig. 2. Mandibular hold on the neck in *Halictus ligatus*.

4. CP (C-Posture): A female curls her abdomen under the thorax so her body forms a C-shape with mandibles and sting pointed at the other female. This behavior is commonly observed in intra- and interspecific interactions in other halictines and is used here for interspecific comparisons (see Fig. 6). An average of two CPs between interacting females of *H. ligatus* occurred in the first 15 min, and one in the next 15 min of the encounters. There were no occurrences of this behavior thereafter in any caste pair. Due to its rare occurrence, we did not consider CP a reliable indicator of dominance in this species.

Avoidance/Withdrawal Behaviors

5. WD (Withdraw): A bee makes a 180° turn away from the other individual. This occurred when the two bees were approximately one body length apart. This behavior suggests avoidance of an encounter and we interpret it as a sign of subordinate behavior.

6. SBR (Synchronized Back and/or Reverse): Both bees do tandem reversals or backing. This behavior was scored mutually when both bees

reversed or backed at the same time or the second did so within 5 s of the first. This mutual avoidance behavior does not permit us to discriminate dominant and subordinate behaviors.

Solicitation/Cooperation Behaviors

7. BNR (Back Without Reverse): A bee backs through the circle tube without reversing. In other species, this movement usually elicits Following (see below) by the other bee. In laboratory nests, it is considered a behavior of solicitation for cooperation by a dominant individual and it is exhibited by queens that lead foragers to brood cells (Breed and Gamboa, 1977). In comparison to studies of other species, *H. ligatus* females Followed subsequent to BNR rather uncommonly. Nevertheless, we interpret BNR as a behavior most likely to be exhibited by the dominant bee.

8. FF (Follow): This is forward movement by a bee toward another that is exhibiting BNR. This is considered a behavior of acquiescence performed predominantly by subordinate bees.

9. PAS (Pass): Forwarding bees meet and maneuver to accommodate each other while they Pass venter to venter in the circle tube. This behavior was usually mutual, that is, both bees were active Passers. If this behavior was not mutual, we scored only for the active Passer. Nonetheless, it is considered a cooperative behavior (Breed *et al.*, 1978; Kukuk, 1992b) because it requires coordination between interactants: it is a behavior in which dominance of one bee over another cannot be determined.

Data Analysis

To examine changes in the frequencies of the eight circle tube behaviors over time, Temporal variation was summed at time intervals of 15 min. Among same-size (foundress–foundress and forager–forager) pairs, bees retroactively designated number 1 were those that engaged in high levels of dominant behaviors (PUL, BNR, and MHN), while those designated number 2 were those that engaged in high levels of the subordinate behavior, WD. Spearman rank correlation tests (Zar, 1984) established the significance of a decline in one behavior and increase in another. Differences between castes in the duration and time of initiation of MHN and MHA during the encounters were tested for significance using the Mann–Whitney *U* test (Sokal and Rohlf, 1995). This test was also used to determine significant differences in size and ovarian development between the interactants where sample sizes were small. Mean differences in size and ovarian development in queen–forager pairs were tested for significance using *t* tests. Chi-square

tests (Zar, 1984) were used to compare the number of bees of a particular caste that engaged in a particular behavior with the number that did not.

RESULTS

Detecting Dominance

The frequency of occurrence of the various behaviors in the first 15 min for all caste pairs is shown in Fig. 3. In six of the nine caste pair types used in our experiments, the individual that was dominant was readily determined within 15 min (Fig. 3). Only in same-size foundress–foundress pairs and paired foragers of the same and different size was it not readily apparent which individual was dominant within 15 min. In four of the six cases, higher frequencies of both PUL and BNR were performed by dominant bees earlier in the encounters and PUL was accompanied by withdrawal (WD) of subordinate bees.

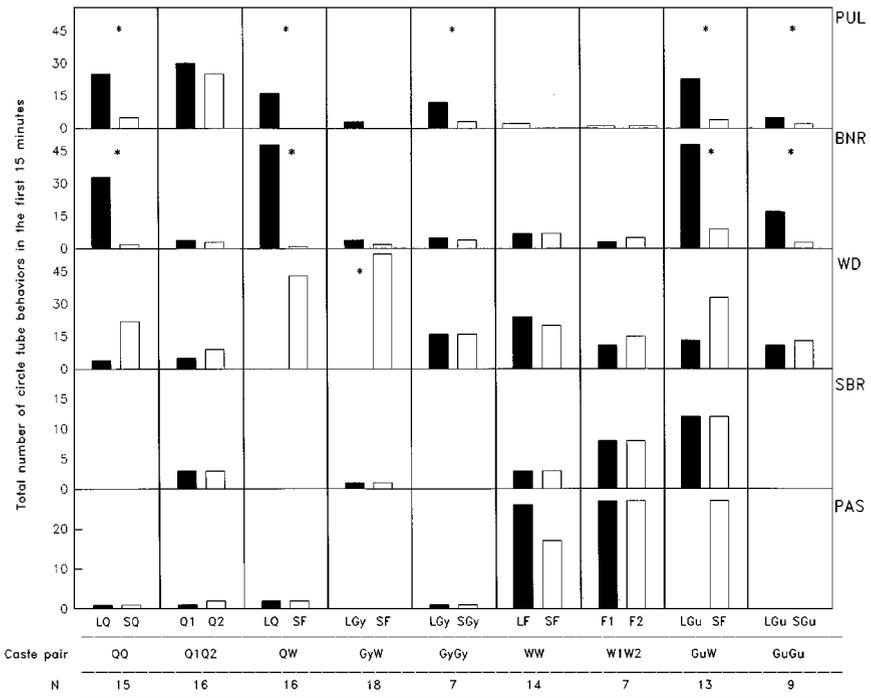


Fig. 3. Comparison of the total number of circle tube behaviors exhibited in the first 15 min by caste pairs of *Halictus ligatus* (N = number of trials).

PUL was a better determinant of dominance in gyne–gyne pairs than BNR, partly because levels of the latter behavior were consistently low throughout most of the encounters. In gyne–forager pairs, PUL and BNR were inadequate for detecting dominance, but marked levels of avoidance (WD) by foragers in the first 15 min allowed us to recognize dominance.

Of the three caste pair types for which dominance could not be determined within the first 15 min, extending the observation period provided unambiguous establishment of dominance in different-size forager–forager and same-size foundress–foundress pairs. Although levels of BNR in the former were very low, smaller foragers exhibited higher frequencies of WD than their larger counterparts after a period of more than half an hour in the circle tubes (Fig. 4G). Levels of WD and BNR in same-size foundress–foundress pairs did not differ markedly between the two interactants, precluding determination of dominance based on these behaviors. After 45 min of interaction, levels of PUL and a sustained aggressive behavior (MHN) enabled us to detect dominance in this caste pair (Figs. 4D and E).

Behavioral differentiation between same-size forager–forager pairs was minimal throughout the observation period, and unlike same-size foundress–foundress pairs, these bees never engaged in MHN and dominance was not determined in these pairs.

Three behaviors (SBR, PAS, and MHA) did not allow us to detect dominance. Levels of SBR and PAS were absent or low in most caste pairs (Fig. 3). However, moderate to high frequencies of both behaviors were observed in forager–forager pairs of all size combinations (Fig. 3). In these pairs, both interactants engaged in mutual and active Avoidance and Passes. In guard–forager pairs, however, foragers were markedly more active Passers than their counterparts, which, in the first 15 min of the encounters, never engaged in this behavior (Fig. 3). We look at MHA as a response to MHN in detail below.

Sustained Aggressive Behaviors

Declining levels of PUL and BNR were accompanied by an increase in the levels of MHN in five caste pair types in which dominance was established within 15 min (for example Figs. 4B and C). MHN was performed by bees that previously exhibited higher frequencies of PUL or BNR and those that withdrew from initial encounters were those that were subjected to MHN. This hold was not observed in (i) forager–forager pairs of either size combination, presumably due to the high levels of avoidance (WD) that persisted throughout the trials (Fig. 4G), and (ii) guard–guard pairs, as they were the least active pairs during the encounters (Fig. 3). The upward trend

of MHN by the larger member of a pair continued to the end of the trials with different-size foundress–foundress (Fig. 4C) and queen–forager pairs (Fig. 4F). In both there were simultaneous declines in PUL and/or BNR (for example, Figs. 4A and B). The significant negative correlations (Table I) of these dominant (PUL and BNR) and subordinate (WD) behaviors with

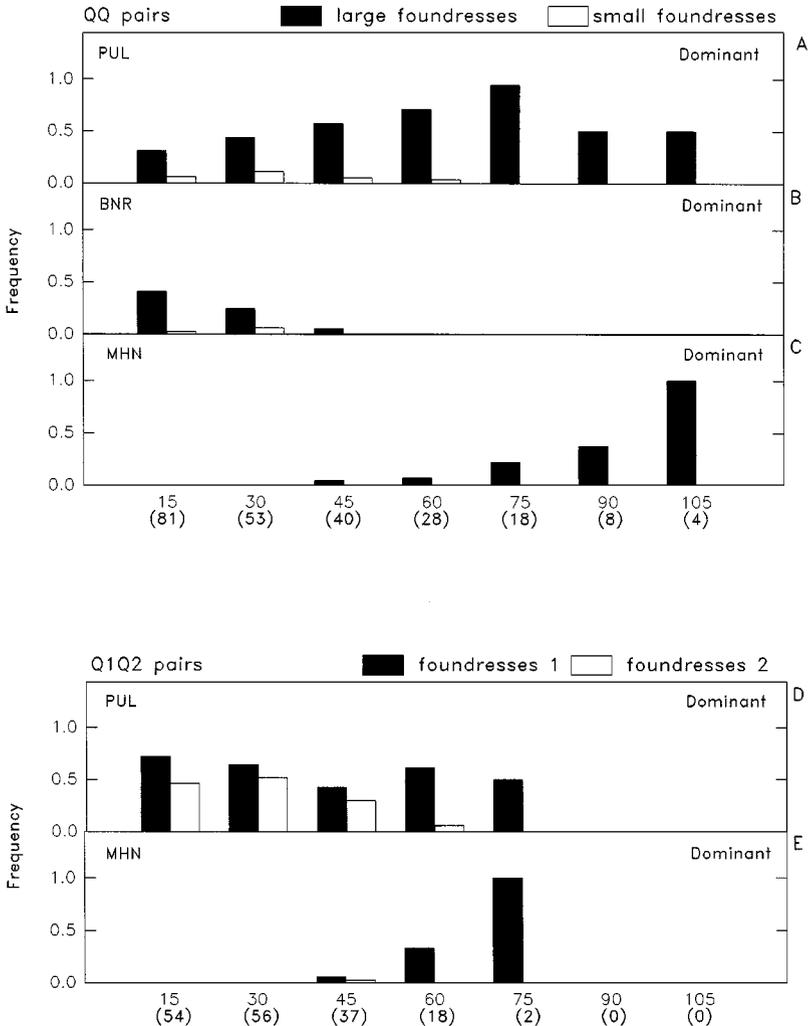


Fig. 4. Mean proportion of circle tube behaviors per frontal encounter among various caste pairs of *Halictus ligatus*. QQ (A–C) and Q1Q2 (D, E); QW (F); WW (G); GyGy (H); GuW (I, J). For acronyms see legend to Fig. 1. Note: Unparenthesized numbers below the x axes indicate time, and numbers in parentheses indicate the total number of frontal encounters.

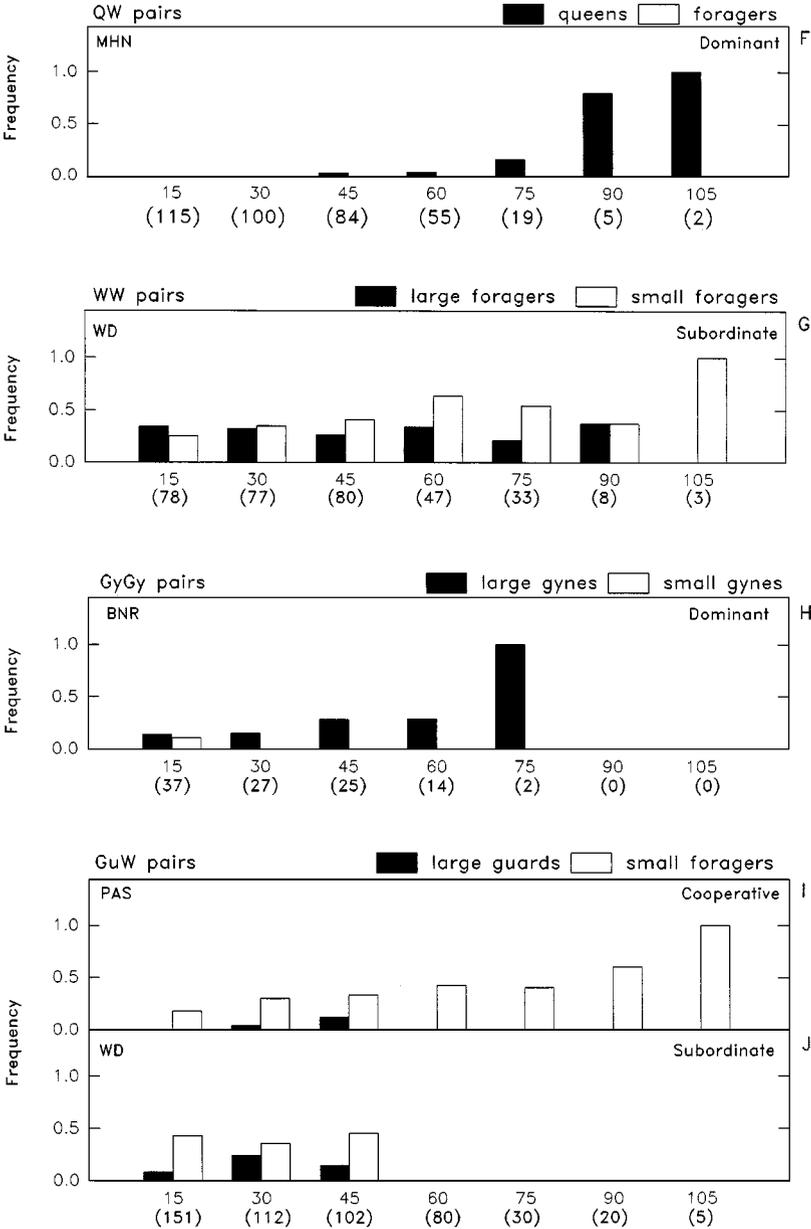


Fig. 4. (Continued)

Table I. Correlation of Initial Dominant–Subordinate Behaviors with MHN (Mandibular Hold on the Neck) in Castes of *Halictus ligatus*^a

Circle tube behavior	<i>r_s</i> for caste pair	
	QQ	QW
	Caste	
	LQ	Q
PUL	–0.908*	–0.838*
BNR	–0.913*	–0.831*
	SQ	W
WD	–0.927**	–0.831*

^aOnly in these caste pairs did levels of MHN continue to increase up to the end of the trials (Spearman rank correlation test). QQ, different-size foundress–foundress pairs; QW, queen–forager pairs; LQ, large foundresses or queens; SQ, small foundresses; Q, queens; W, small foragers; PUL, Push and Lunge; BNR, Back Without Reverse; WD, withdraw; *r_s*, coefficient of rank correlation. *Significant at 0.05 level; **significant at 0.01 level.

MHN suggest a behavioral switch in dominant bees from behaviors that permit withdrawal by the subordinate to those that prevent escape during the encounters. In both pairs, this switch allowed the large bees to remain dominant throughout the encounters.

The highest percentage of individuals that initiated MHN were large members of foundress–foundress and gyne–gyne pairs (Table II). Initiation rates and durations differed significantly between these two groups (Fig. 5), with large gynes initiating MHN earlier than large foundresses (Mann–Whitney *U* test, $U_s = 119$, $P < 0.005$). However, the latter sustained this hold longer than the former ($U_s = 125$, $P < 0.001$). It should be stressed, however, that although incidences of MHN were relatively low compared to PUL in all caste pairs, this hold was sustained by aggressor bees for relatively long periods of time (up to 45 min) during the encounters. Three foragers (in queen–forager pairs) were decapitated and the head of a smaller foundress (in different-size foundress–foundress pairs) was twisted 180° as a result of this prolonged hold. Although there were significant size differences between the killed foragers and their queen counterparts (mean head width difference = 0.56 mm; $U_s = 9$, $P < 0.05$), the difference in ovarian development between them was not quite significant (mean ISO difference = 8; $U_s = 8.5$, $P = 0.08$). In this caste pair, the size and ovarian development of

Table II. Size Differences Between Individuals of *Halictus ligatus* that Engaged in MHN (LQ, LGy, LGu) and Bees that Were Recipients of MHN (SQ, SF, SGy) and the Percentage of Pairs in Which MHN and MHA Occurred (*P* Values for Each Caste Pair Type Are All <0.001)^a

Caste pair type (<i>N</i>)	Bees that engaged in MHN		Mean % diff. in head width	<i>t</i>	df	% of pairs in which MHN and MHA occurred	% of MHN recipients that responded with MHA
	Number	Percentage					
QQ (15)	14	93.3	17.3	9.55	26	20.0	21.4
QW (16)	13	81.2	17.9	8.45	24	50.0	61.5
GyGy (7)	7	100.0	15.0	4.19	12	100.0	100.0
GyW (18)	10	55.6	13.8	6.07	18	00.0	00.0
GuW (13)	8	61.5	12.5	6.48	14	61.5	100.0

^aQQ, foundress pairs; QW, queen–forager pairs; GyGy, gyne–gyne pairs; GyW, gyne–forager pairs; GuW, guard–forager pairs; LQ, large foundresses or queens; SQ, small foundresses; SF, small foragers; LGy, large gynes; SGy, small gynes; LGu, large guards; *N*, number of circle tube trials.

surviving foragers were significantly less than those in their queen counterparts ($n = 13$; mean head width difference = 0.62 mm; $t = 8.814$, $P < 0.001$; mean ISO difference = 4.69; $t = 5.668$, $P < 0.001$). This suggests that queens become extremely aggressive only toward foragers with comparatively well-developed ovaries.

Three of seven small gynes in gyne–gyne and 2 of 13 small foragers in guard–forager pairs subjected their larger counterparts to MHN between 30 and 45 min of the encounters, after which these smaller bees were subjected to the same mandibular hold in the next 45 min. Between 45 and 60 min, most large gynes (four of seven) in gyne–gyne pairs engaged in MHN, while their counterparts (four small gynes) engaged in MHA a few seconds later. By the 75th min, all seven large gynes had engaged in MHN and all seven of their counterparts (small gynes) had engaged in MHA (Table II). Although MHN and MHA were displayed independently, it is important to emphasize in these pairs that when one gyne engaged in MHN, the other always responded by engaging in MHA, suggesting that this response may be defensive in this context.

In different-size caste pairs, the bees that engaged in MHN were on the average 1.17 times larger than their smaller counterparts (Table II). Even with the significant differences between larger bees and their smaller counterparts in each of the five caste pairs (Table II), there was variation in the number of throttled bees that responded with MHA. All small gynes in gyne–gyne and small foragers in guard–forager pairs fought back, while none of the MHN recipients (foragers) in gyne–forager pairs retaliated against their gyne counterparts (Table II). There were no significant differences in size between the small foundresses that fought back and those that did not (mean head

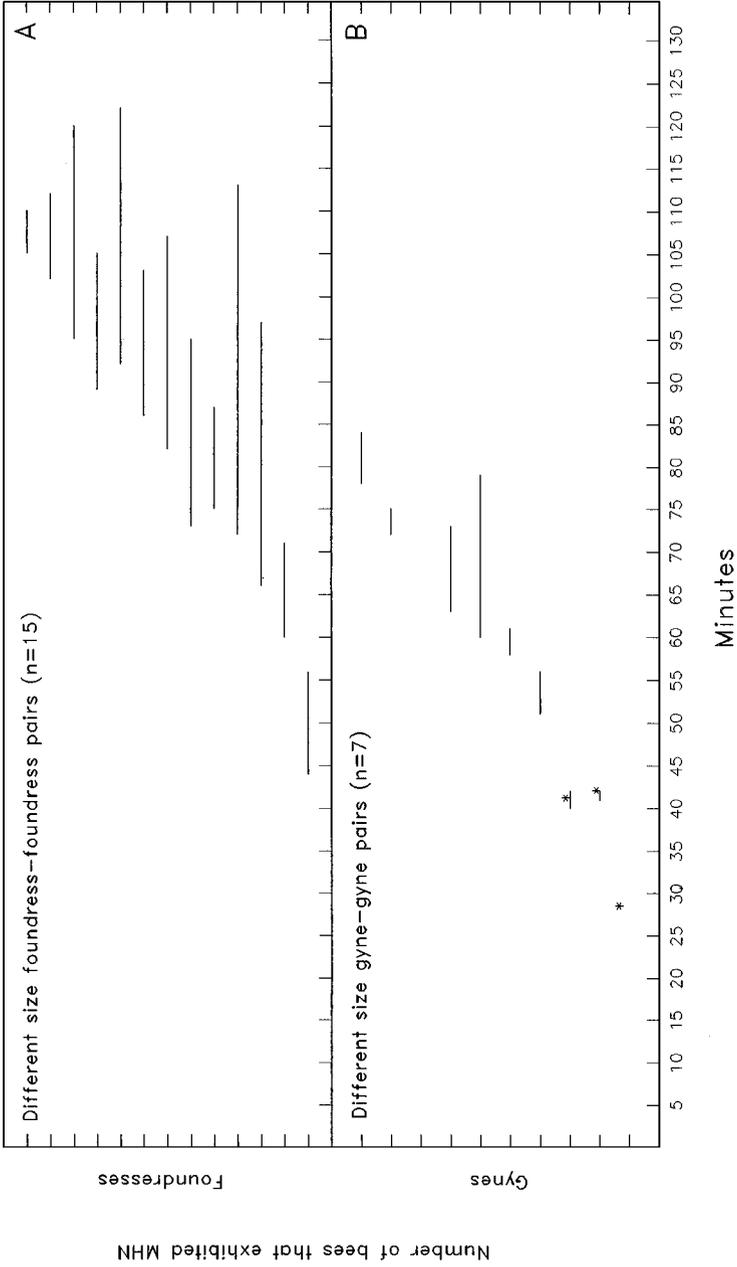


Fig. 5. Durations of Mandibular Hold on the Neck (MHN) in different-size foundress–foundress and gyne–gyne pairs of *Halictus ligatus*.

width difference = 0.08 mm; $U_s = 14$, $P > 0.10$; $n = 3$ and $n = 11$, respectively). The foragers that retaliated against their queen were significantly larger than those that did not fight back (mean head width difference = 0.22 mm; $t = 3.38$, $df = 14$, $P = 0.005$; $n = 8$), although they were still significantly smaller than their adversaries (mean head width difference = 0.41 mm; $t = 6.06$, $df = 14$, $P < 0.001$).

DISCUSSION

First, we discuss our findings in relation to the social biology of the species investigated, then we discuss the relevance of the results from *Halictus ligatus* to circle tube experiments with other halictines and compare the results obtained among taxa.

Our initial objectives were to determine (i) the time required and (ii) the behaviors most readily used in the detection of dominance of one individual over another, and (iii) to see whether behavior patterns within the circle tubes varied after extended periods of time.

Clearly the traditional 15-min duration of circle tube experiments was sufficient for the determination of dominant and subordinate behavior in most caste pairs used in our experiments (Table III). However, extension of this duration to more than 30 min was necessary for different-size forager–forager pairs and same-size foundress–foundress pairs, and we were not able

Table III. Summary of Dominance Behaviors in *Halictus ligatus*^a

Caste pair type	Time required to detect dominance (min)	Behaviors best used to detect dominance	Switch to MHN by dominant bees	Time of initiation of the first MHN by dominant bees (min)	Retaliated with MHA by subordinate bees
QQ	15	PUL, BNR	Yes	43	Yes
Q1Q2	45	MHN	—	46	Yes
QW	15	PUL, BNR	Yes	41	Yes
GyW	15	WD	Yes	26	No
GyGy	15	PUL	Yes	52	Yes
WW	>30	WD	No	na	No
W1W2	∞	None	No	na	No
GuW	15	PUL, BNR	Yes	55	Yes
GuGu	15	PUL, BNR	No	na	No

^aQQ, different-size foundress–foundress pairs; Q1Q2, same-size foundress–foundress pairs; QW, queen–forager pairs; GyW, gyne–forager pairs; GyGy, gyne–gyne pairs; WW, different-size forager–forager pairs; GuW, guard–forager pairs; GuGu, guard–guard pairs; MHN, mandibular Hold on the Neck; MHA, mandibular Hold on the Appendages; PUL, Push and Lunge; BNR, Back Without Reverse; WD, Withdraw.

to determine which individual was dominant in same-size forager–forager pairs even when observations were extended to 90 min.

The behaviors that were most useful in distinguishing dominant from subordinate bees also varied according to caste affiliations of the interactants (Table III). PUL by dominant individuals, often accompanied by WD by the subordinates, and BNR, also by dominant individuals, were the behaviors most likely to differentiate the two bees. In the more serene gyne–forager interactions, high levels of WD appeared to indicate subordinate status. WD also enabled us to discriminate between interactants in different-size forager–forager pairs but only after an extended period of observation. Sustained aggressive behaviors arising late in the encounters were the only way in which we could identify dominant individuals in same-size foundress–foundress pairs.

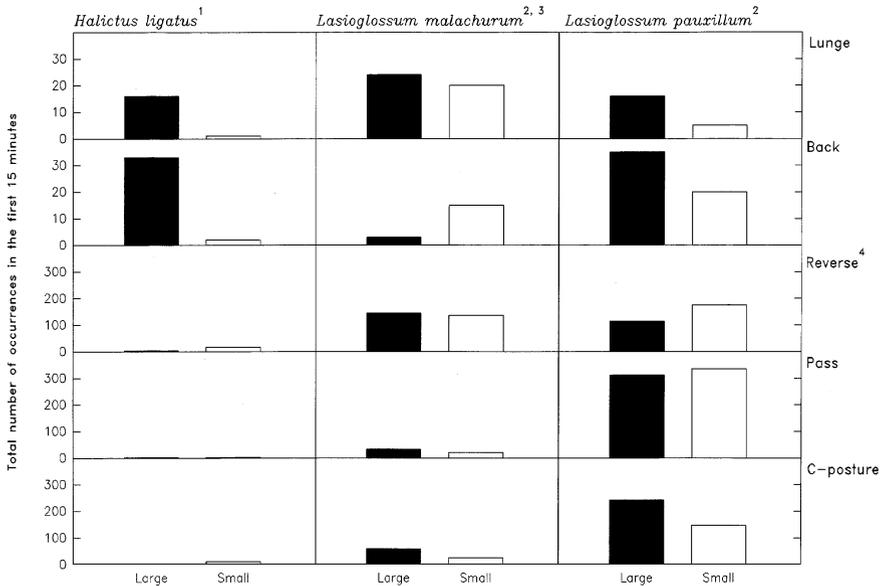
In many instances the caste-based differences in behavioral frequencies make sense based upon the roles of the bees within the society. Queens and gynes were the more aggressive individuals, as might be expected. Foragers were more likely to pass other individuals, and guards were rarely observed being active participants in Passes but were frequently passed—this difference is expected given the role of guards at nest entrances, permitting entry and egress of forager nestmates but not of other individuals or heterospecifics. Even the relative ease with which we could detect which individuals were dominant is generally comprehensible given an understanding of the behavior of bees under natural conditions. One might expect bees of different caste or of different sizes within a caste to establish dominance relationships more readily than those of the same size and caste. Foragers are more likely to be recipients of dominant behaviors from queens and to interact less among themselves than other caste combinations in normal circumstances, and so it is perhaps not surprising that same-size forager–forager pairs constituted the sole instance in which we were unable to identify the dominant individual.

The most important finding that resulted from the extended period of observation was the switch to sustained aggression in some caste pairs. This switch occurred in all pairings involving queens or gynes and also by guards in guard–forager pairs. In the latter pairs, switching by guards to sustained aggression in the middle of the encounters eliminated WD by their counterparts (small foragers) (Fig. 4J). MHN was most marked in foundress–foundress pairs in which such aggression permitted dominance relationships to be detected in the absence of any marked difference between interactants earlier in the experiments. This switch seems to signal a change from behaviors which permit to those that preclude the escape of the subordinate individual. In all instances except foragers in gyne–forager pairs, MHN was accompanied by retaliatory MHA. Such behavioral responses may have been attempts to disengage from MHN or reduce its effects.

The most surprising result of our study was the observation of four examples of one bee killing another; in three instances it was a forager daughter nestmate of a queen which was killed by her mother. This would seem to be difficult to explain as being part of normal behavior, and it is possible that it merely represents a pathological response to unnatural conditions analogous to some behaviors observed in zoo animals (Hediger, 1964; Packard *et al.*, 1990). However, dead bees are commonly found during nest excavations of halictines (Packer, personal observation; Weislo, personal communication), and it is possible that some of them may have been killed by a nestmate *in situ* rather than having died of another cause such as old age or parasitism. The fact that the killed daughters had ovaries as well developed as those of their mothers is consistent with the hypothesis that higher levels of aggression are aimed at individuals that pose a greater threat to the queens direct reproductive success. Nonetheless, nestmates of this species have been observed to treat one another very roughly both in laboratory observation nests (Packer, 1986a) and at nest entrances in the field (M. Richards, unpublished observation). The most commonly observed aggressive interaction is of queens attempting to eject workers from the nest, as though in an attempt to get them to forage. It is possible that the inability of foragers to leave the circle tube arena elicited the escalated aggressive response of their mothers; nonetheless, the actual killing of workers seems excessive and is likely due at least partly to the artificiality of the circle tube setting. In the field, *L. malachurum* gynes immediately depart when their limbs (antennae or legs) are bitten off by nest usurpers. Even in this setting where escape is possible, killed bees have been found in areas adjacent to nest entrances where fighting occurred (Smith and Weller, 1989). Such highly aggressive behaviors have never been reported in any of the numerous laboratory observation studies of *L. zephyrum*.

Our study indicates that it is only the sustained aggressive behaviors that may not be detected in the comparatively short circle tube experiments that typify most studies (Breed *et al.*, 1978; Smith and Weller, 1989; Kukuk, 1992; McConnell-Garner and Kukuk, 1997; Weislo 1997; Paxton *et al.*, 1999). Extending observations to over 1 h in *L. zephyrum* failed to elicit similar aggressive behavior (Pabalan, unpublished observation), and so such intense interactions may not be typical of other halictine genera but are perhaps worthy of further investigation by prolonged observations of other taxa.

Behavioral differentiation between different-size foundresses is more acute in *H. ligatus* than in either of the *Lasioglossum* (Evylaeus) species for which data are available (Fig. 6) (Smith and Weller, 1989). The low frequency of passing by foundresses in *H. ligatus* in comparison to that by foundresses of other species (Table IV) is also notable, although the frequency of this behavior by foragers is similar to that for both queens and foragers of *L. zephyrum*



NOTE: 1) Behavioral data were obtained from the first 15 minute interactions between different-size foundress-foundress pairs

2) Data are from Smith and Weller (1989)

3) The bees were subjects of a pheromone experiment and as part of the controls, the small individuals were acetone-treated

4) Behavioral term used for *Halictus ligatus*. Smith and Weller (1989) used the term back away

Fig. 6. Comparison of the total number of occurrences of circle tube behaviors in different-size spring queens (gynes) among three species of primitively eusocial bees.

and the solitary species (Table IV). The cooperative nature of communal species (Table IV) is indicated by their high level of Passing (McConnell-Garner and Kukuk, 1997; Paxton *et al.*, 1999), and this contrasts with the competitive interactions that are increasingly being seen as typifying interactions among individuals in eusocial taxa. Less readily comprehensible is the low frequency of withdrawals per encounter in *H. ligatus* compared to that in other species (Table IV). Only in the communal *L. hemichalceum* is a similarly low withdrawal rate found. The behavior of communal species is the least aggressive and that of *H. ligatus* the most aggressive of any species studied using the circle tube method. It appears that not only do *H. ligatus* females interact aggressively, but also they do not avoid such encounters as readily as do other eusocial or solitary halictines in the circle tube setting.

Halictus ligatus has a very wide head, which varies allometrically such that large individuals have disproportionately massive heads (Knerer, 1980), especially behind the compound eyes in the area where the mandibular adductor muscles arise. It also has very long mandibles (indeed, larger individuals have a bite that is almost worse than the sting to a human being). It seems

Table IV. Percentage of Trials in Which Pass (PAS) Occurred and the Mean Proportion of Withdraw (WD) per Encounter (E) in Halictine Species of Differing Social Organizations^a (*N* = Number of Circle Tube Trials)

Species	<i>N</i>	Pass	WD/E
Primitively eusocial			
<i>H. ligatus</i> ^b			
Foundresses	31	6.4	0.183
Foragers	21	66.7	0.133
Guards	9	00.0	0.231
<i>L. zephyrum</i> ^c			
Queens	11	54.5	0.451
Foragers	22	54.5	0.516
Guards	12	00.0	0.915
Communal			
<i>L. hemichalceum</i> ^d	26	96.1	0.153 ^d
<i>Andrena scotica</i> ^e	40	45.0	0.650 ^f
<i>Panurgus calcaratus</i> ^e	24	91.7	0.320
Solitary			
<i>L. platycephalum</i> ^g	17	58.8	0.548
<i>L. (Ctenonomia) sp</i> ^g	24	54.2	0.610
<i>L. figueresi</i> ^h			
Provisioning bees	20	60.0	0.480
Active bees	15	50.0	0.600
Inactive bees	14	85.7	0.546

^aPercentage of Pass and number of WD/E within the first 10 min of interactions in *H. ligatus* were compared with those of other species, the entire duration of which spanned 10 min.

^bFoundress pairs of different and same sizes were combined, as were different and same size forager pairs. Guard pairs are of different sizes.

^cData for *L. zephyrum* from Breed *et al.* (1978).

^dKukuk (1992) used the term Avoidance for *L. hemichalceum*.

^eData for *Andrena scotica* and *Panurgus calcaratus* from Paxton *et al.* (1999).

^fWD/E, withdrawal per frontal encounter among nonnestmate bees.

^gData for *L. platycephalum* and *L. (Ctenonomia) sp.* from McConnell-Garner and Kukuk (1997).

^hData from Weislo (1997).

likely that these enlarged structures arose as a result of selection for success in intraspecific interactions such as those we have described. Based upon the general scarcity of such extreme morphological adaptations, we would not expect many other halictine species to exhibit such escalated levels of aggression. Other species with enlarged heads and long mandibles which might be similarly antagonistic include *H. (H.) scabiosae*, *H. (H.) modernus* (Knerer, 1980; Packer, personal observation), *H. (H.) latisignatus* (Sakagami and Wain, 19??), and especially the queens of *H. (Seladonia) lanei* (Janjic and Packer, unpublished observation).

In summary, circle tube experiments on *H. ligatus* demonstrate that dominance relationships can be detected within 15 min of observation for most caste pairs but that different behaviors typify dominance interactions in different caste combinations. Particularly aggressive behaviors are not observed unless the duration of observation is extended substantially, and it seems that the level of agonism in this species is much higher than that in *L. zephyrum*. North temperate populations of *Halictus ligatus* generally have small colony sizes, poor morphological and physiological caste differentiation, and intense intracolony conflict over oviposition (Packer, 1987; Richards *et al.*, 1995). The apparently intensely competitive interactions among *H. ligatus* females are mirrored in both their morphology (very long mandibles and heavy mandibular adductor musculature) and their reproductive status, with high levels of worker ovarian development and substantial amounts of ovarian resorption in both castes (Packer, 1987; Pabalan, in preparation); workers are also capable of laying diploid in addition to haploid eggs (Richards *et al.*, 1995). Considerably more research is needed to determine how unusual our observations of *H. ligatus* are in comparison to behaviors in other social halictines, nonetheless, it seems clear that the circle tube arena is a useful tool in such investigations.

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